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Cell adhesion mediated by a purified fucosyltransferase

(fibroblast spreading/glycosyltransferases/cell surface carbohydrates)

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ABSTRACT **Human embryonic skin fibroblasts attach and spread on surfaces on which a fucosyltransferase purified from human milk has been immobilized. The adhesion-enhancing effect of the transferase involves specific interactions of the enzyme surface with the cell surface carbohydrate acceptors, as suggested by the following findings. About 80% of human embryonic skin fibroblasts attach and spread in 1 hr on fucosyltransferase surfaces; in contrast, bovine serum albumin, fetuin, asialofetuin, and asialotransferrin surfaces fail to enhance adhesion. The adhesion-mediating activity of the transferase is destroyed by alkylation of the sulfhydryl groups or by heating. The adhesion on fucosyltransferase surfaces is inhibited by glycoprotein, glycolipid, and oligosaccharide acceptors containing the sugar sequence galactosyl-(β1 → 4)-*N*-acetylglucosamine, in agreement with the substrate specificity of the enzyme. The results suggest that glycosyltransferases are able to stimulate cell adhesion in a manner similar to that proposed for lectins.**

Knowledge of molecular mechanisms of cell–cell and of cell–substratum recognition is of fundamental importance when attempts are made to understand the origin of supracellular order found in multicellular organisms. Little knowledge exists concerning the type of interactions that should be considered as the molecular basis of specific cell adhesion.

There is increasing evidence that protein–sugar interactions could mediate specific cell recognition (1–3). This inference is based on the facts that the sugar moieties of proteins and lipids are located at the outer surface of membranes (4) and that carbohydrate-binding proteins are often found as surface-exposed components of the cell (2). Furthermore, carbohydrate-reactive proteins have been shown to mediate cell adhesion in model systems (5–7).

Lectin-type activities are often considered as mediators of specific cell adhesion (2). It was recently shown that galactose oxidase and some glycosidases immobilized on plastic can also give sufficient affinity for a few cell types to trigger a specific adhesion reaction (5). A high α-mannosidase activity was also demonstrated at the surface of a fibroblastic cell (7).

The occurrence of glycosyltransferases as cell surface-exposed components has been suggested by Roseman (8). According to this hypothesis, glycosyltransferases could mediate specific cell adhesion. However, no direct adhesion studies supporting the glycosyltransferase hypothesis have been presented. This may be due to the fact that glycosyltransferases have been purified only recently (9), and sufficient quantities of the enzymes are still hard to obtain.

The glycosyltransferase hypothesis was tested with the purified *N*-acetylglucosaminide-α1,3,α1,4-fucosyltransferase of human milk (10). This was possible by using a model system that requires only low amounts of protein (5). The results of this study suggest that the transferase immobilized on plastic surfaces can induce attachment and spreading of cells in a way that is dependent on the specificity of the enzyme.

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MATERIALS AND METHODS

Materials. *N*-Acetylglucosaminide-α1,3,α1,4-fucosyltransferase was purified from human milk as described (10). Fibronectin was purified from human plasma according to Vuento and Vaheri (11). Fetuin (type III), transferrin, and crystalline bovine serum albumin were purchased from Sigma. Asialofetuin and asialotransferrin were prepared from fetuin and transferrin by weak acid hydrolysis (0.1 M HCl, 80°C, 1 hr) followed by dialysis against phosphate-buffered saline (P_i/NaCl). Paragloboside was prepared by preparative thin-layer chromatography from a neuraminidase-treated sialylparagloboside of human kidney (12). *N*-Acetyllactosamine was prepared from its sialylated form by weak acid hydrolysis (13). A mixture of lacto-*N*-tetraose (77%) and lacto-*N*-neotetraose (23%) was isolated from human milk (14).

Adhesion Assays. Human embryonic skin fibroblasts were cultured in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum, 100 units of penicillin G per ml, and 0.1 mg of streptomycin per ml in an atmosphere of 5% CO_2/95% air. Confluent cell monolayers were dispersed with crystalline trypsin (Sigma), 10 μg/ml in Ca^{2+}- and Mg^{2+}-free P_i/NaCl, and washed in the presence of soybean trypsin inhibitor (40 μg/ml) as described (5). The cells were washed free of soybean inhibitor by two centrifugations in P_i/NaCl or in Hepes-buffered saline (5). The single-cell suspensions were used in the adhesion assays within 0.5 hr after their preparation.

Fucosyltransferase was adsorbed on Linbro polystyrene plates (microtiter 96-well plates) at 10 μg/ml in 50 μl of 20 mM 3-(*N*-morpholino)propanesulfonic acid NaOH, pH 7.5/5 mM $MnCl_2$/20 mM NaCl/50% (vol/vol) glycerol for 1.5 hr at room temperature. Then, the medium was removed and the plates were treated with bovine serum albumin (100 μg/ml in 100 μl of P_i/NaCl) for 1 hr at room temperature. The coating medium was removed, the plates were washed three times with 150 μl of P_i/NaCl, and 50 μl of P_i/NaCl or Hepes-buffered saline (5), as specified in the text, was applied to the plates.

The assays were started by adding $2.50–3.75 \times 10^4$ cells in 50 μl of the same medium. The plates were incubated at 37°C for 1 hr. The reactions were stopped by adding 100 μl of 4% glutaraldehyde in P_i/NaCl, and the proportion of cells spread was counted from randomly selected areas on the wells. All values are based on counting of 250 cells if not otherwise indicated in the text. No attempt was made to estimate different stages of the spreading, but all cells that had lost their round shape were considered as spread. In some experiments the nonadherent cells were washed off by rinsing the wells three

Abbreviation: P_i/NaCl, phosphate-buffered saline.

times with 150 μl of P_i/NaCl before fixation with glutaraldehyde in order to estimate the degree of cells bound on the plates. Analysis of cell spreading was preferred over a simple binding analysis because, even after the washing, fibroblasts with a round shape (which may not represent truly adhering cells) were often seen bound to the surfaces.

Analytical Methods. Protein was determined according to Lowry *et al.* (15). Fucosyltransferase was assayed with lactose as an acceptor (10). Triton CF-54 (0.5%) was included in the incubation mixtures in order to facilitate analysis of the enzyme activity adsorbed to the polystyrene plates.

RESULTS

Cell Attachment and Spreading on Fucosyltransferase Surfaces. Human embryonic skin fibroblasts were able to attach and spread on surfaces coated with the purified fucosyltransferase from human milk (Fig. 1). The effect could be observed at 1 μg of the transferase per ml of coating solution, and the maximal effect (80% attachment and spreading) was achieved at 6–10 μg/ml. Under these conditions the enzyme activity measured from the surfaces corresponded to 5% of the activity used for coating. Under the same kind of conditions, the coating buffer itself, fetuin, asialofetuin, asialotransferrin (Fig. 1), and bovine serum albumin (Fig. 2) had little or no adhesion-enhancing effect.

Kinetic evaluation of the transferase-mediated adhesion (10 μg/ml) revealed a slower rate than cell adhesion on concanavalin A (Fig. 2). However, after 60 min, about 80% of the cells were attached and spread on both surfaces. In contrast to concanavalin A and fucosyltransferase surfaces, no reaction was observed on surfaces coated with bovine serum albumin (100 μg/ml), which were studied in the same experiment up to 100 min.

In an attempt to study whether an increase in the acceptor density at the cell surface could increase the intensity of adhesion (7, 16), the cells were treated with *Vibrio cholerae* neuraminidase, and then the enzyme was removed by repeated centrifugations of the cells. It was found that most of the adhesion activity was lost from both the enzyme-treated and control cells during this treatment. However, the cells treated with neur-

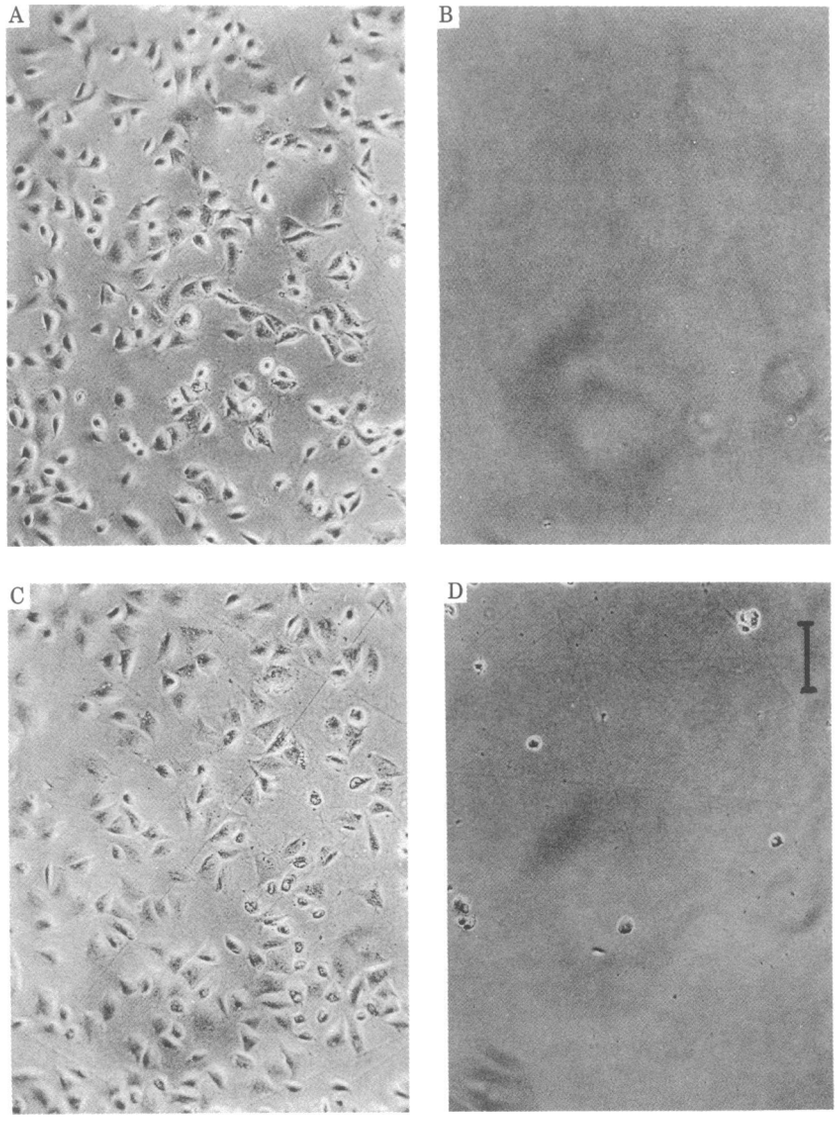

FIG. 1. Attachment and spreading of human embryonic skin fibroblasts on different protein-coated surfaces. The wells were tested for adhesion activities by adding 2.5×10^4 cells in P_i/NaCl. The plates were incubated for 1 hr at 37°C, and the nonadherent cells were washed off by rinsing the wells three times with 150 μl of P_i/NaCl. (*A*) Fucosyltransferase; (*B*) fucosyltransferase with asialotransferrin in the medium at 0.1 mg/ml; (*C*) fibronectin (coated at 10 μg/ml); (*D*) asialotransferrin (coated at 100 μg/ml). (Bar = 100 μm.)

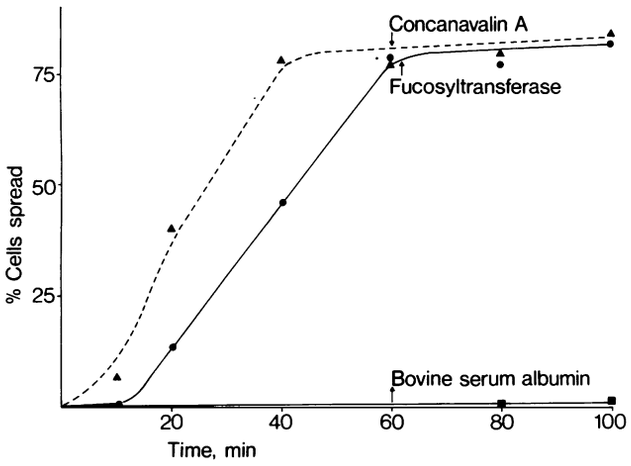

FIG. 2. Kinetics of cell spreading on surfaces coated with fucosyltransferase (●), concanavalin A (▲), or bovine serum albumin (■). Fucosyltransferase was coated on the wells as explained in *Materials and Methods* but post-coating with albumin was omitted. Concanavalin A was coated at 6 μg/ml and bovine serum albumin was coated at 100 μg/ml in P_i/NaCl. Human embryonic skin fibroblasts (2.5×10^4 cells in P_i/NaCl) were added to different surfaces and incubated at 37°C; the spreading was stopped by adding glutaraldehyde at the times indicated. The percentage cells spread was measured by scoring 150 cells.

aminidase were spread to a somewhat higher extent than the cells treated in the same way without the enzyme. The percentages of cells spread on fucosyltransferase surfaces after 20 and 50 min of incubation were 2.4% and 18% for neuraminidase-treated cells and 0.6% and 8.6% for control cells.

Effect of Enzyme Inactivation on the Adhesion Activity. Heating of the fucosyltransferase to 80°C for 15 min strongly decreased the adhesion-mediating activity (Table 1). Similarly, treatment of the enzyme-coated surfaces with *N*-ethylmaleimide abolished the spreading activity (Table 2). These findings are in agreement with the known sensitivity of the transferase to heating and to *N*-ethylmaleimide (10). In contrast, there was little or no effect on fibronectin-mediated spreading (Table 2), in agreement with the known insensitivity of fibronectin adhesion to sulfhydryl alkylation (17).

Inhibition of Fucosyltransferase-Mediated Cell Adhesion by Specific Acceptors of the Enzyme. When asialotransferrin was included in the medium at 100 μg/ml, a strong inhibition of cell attachment was evident (Fig. 1). The same kind of effect could be observed in a different assay that avoids the washing procedures of the nonadherent cells. In this assay, the cells were incubated on fucosyltransferase surfaces in the presence of different concentrations of asialofetuin and asialotransferrin. The adhesion reaction was stopped by glutaraldehyde, and the proportion of cells spread was counted. A strong inhibition was observed at 50–100 μg of asialofetuin and asialotransferrin per ml of incubation medium (Fig. 3; Table 3). In contrast, sialylated glycoproteins displayed little effect on fucosyltransferase-mediated adhesion. This difference is in agreement with the substrate specificity of the enzyme (10).

Table 1. Effect of heating on cell-spreading activity of fucosyltransferase

Adhesion surface	Cells spread	
	No.	% of control
Native enzyme	58; 61	100
Heated enzyme	12; 16	24

Fucosyltransferase was heated (80°C, 15 min) in the glycerol-containing buffer and coated on the surfaces. Adhesion-enhancing effects of the surfaces were tested by adding 3.75×10^4 cells in P_i/NaCl. The percentage of cells spread was counted from duplicate wells after 1 hr at 37°C.

Table 2. Effect of *N*-ethylmaleimide treatment on the cell-spreading activity of fibronectin and fucosyltransferase surfaces

Adhesion surface	Cells spread	
	No.	% of control
Fibronectin:		
Control	88; 88; 87	100
Treated	81; 63; 53	75
Fucosyltransferase:		
Control	66; 58; 53	100
Treated	6; 4; 2	7

The surfaces were coated with fibronectin (10 μg/ml in P_i/NaCl) or fucosyltransferase, washed, and treated with 100 μl of 30 mM *N*-ethylmaleimide in P_i/NaCl for 30 min at room temperature. The control wells were incubated with P_i/NaCl. The wells were washed three times with P_i/NaCl and tested for adhesion activities by adding 2.5×10^4 cells in P_i/NaCl to triplicate wells.

Whether the inhibition of cell adhesion by asialoglycoproteins is due to a direct binding to the enzyme or to some unknown factor was studied by changing the adhesion surface. Asialofetuin was not effective as an inhibitor of fibronectin-mediated spreading (Fig. 3). At the highest concentrations studied, asialofetuin did not inhibit fibronectin surfaces, even at about 1,000-fold the concentration inhibiting fucosyltransferase.

Interestingly, another polyvalent fucosyltransferase acceptor, paragloboside (Gal-GlcNAc-Gal-Glc-Cer), was effective at about the same concentration of acceptor sequences as the asialoglycoproteins. The data in Fig. 4 suggest that paragloboside is an effective inhibitor at concentrations as low as 5–10 μM glycolipid, which would give approximately the same acceptor concentration as asialofetuin at 100 μg/ml. In contrast to paragloboside, globoside (GalNAc-Gal-Gal-Glc-Cer) was not effective as an inhibitor of fucosyltransferase-mediated cell spreading even at 100 μM (63% of cells spread).

Compared to glycoprotein or glycolipid, higher concentrations of simple oligosaccharides were needed to inhibit the spreading stimulated by fucosyltransferase. The minimal struc-

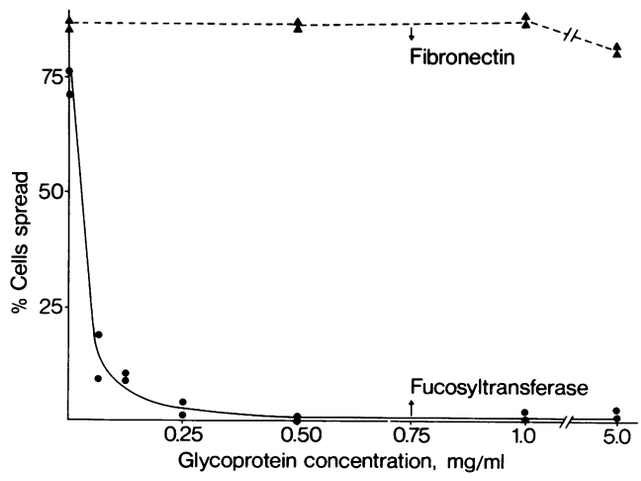

FIG. 3. Effect of asialofetuin on cell spreading. The wells were treated with fucosyltransferase (●) or fibronectin (10 μg/ml) (▲) and then washed; asialofetuin at 2 times the final concentrations in Hepes-buffered saline was added to the wells. Adhesion activities were measured by using 3.75×10^4 fibroblasts in Hepes-buffered saline.

Table 3. Effects of monosaccharides, oligosaccharides, and glycoproteins on the cell-spreading activity of fucosyltransferase

Addition	Concentration used	Cells spread	
		No.	% of control
Monosaccharides and oligosaccharides:			
None		89; 92	100
NeuAc	5 mM	80; 82	89
Gal	5 mM	89; 92	100
GlcNAc	5 mM	88; 88	97
Gal(β1-4)GlcNAc	5 mM	24; 22	25
NeuAc(α2-3)Gal(β1-4)GlcNAc	5 mM	83; 84	92
Gal(β1-3,4)GlcNAc(β1-3)Gal(β1-4)Glc	20 mM	12; 9	12
Glycoproteins:			
None		71; 76	100
Fetuin	1 mg/ml	67; 70	93
Asialofetuin	0.1 mg/ml	9; 10	13
Transferrin	0.1 mg/ml	75; 77	103
Asialotransferrin	0.1 mg/ml	6; 3	6

The wells were treated with fucosyltransferase and then washed; monosaccharides, oligosaccharides, or glycoproteins at 2 times the final concentrations were applied to the plates in Hepes-buffered saline. Controls were incubated with Hepes-buffered saline. After 5 min 2.5×10^4 cells were added, and the percentage of cells spread was analyzed from duplicate wells.

ture required by the enzyme, Gal(β1-4)GlcNAc, was effective at millimolar concentrations. However, the sialylated form of the disaccharide or any of the monosaccharide components of the oligosaccharides tested was not effective (Table 3). A somewhat higher concentration of tetrasaccharide acceptors was necessary for an effective inhibition (Table 3), which may be due to a higher K_m value of the tetrasaccharides compared to the disaccharide (10). Cell spreading on fibronectin surfaces was not inhibited by the concentration of the tetrasaccharides used in the assay, suggesting an effect that is specific for the transferase surface.

DISCUSSION

We propose that the adhesion-enhancing activity of the fucosyltransferase is due to the acceptor specificity of the enzyme. This inference is due to the following findings. A highly purified enzyme triggers cell attachment and spreading that is comparable to that caused by concanavalin A and fibronectin. In contrast, coating of the surfaces with bovine serum albumin, fetuin, asialofetuin, or asialotransferrin fails to enhance adhesion. Heating of the enzyme or treatment with *N*-ethylmaleimide greatly reduces the ability of the transferase to mediate adhesion. Cell adhesion on fucosyltransferase surfaces is specifically inhibited by acceptor structures of the enzyme that contain the sugar sequence Gal(β1-4)GlcNAc.

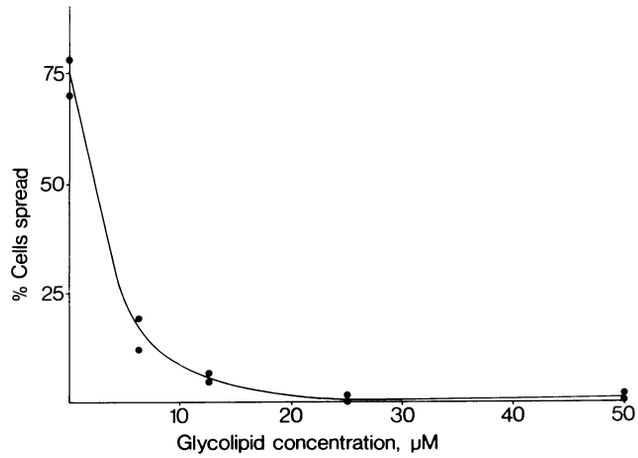

FIG. 4. Effect of paragloboside on fucosyltransferase-induced cell spreading. Paragloboside at 2 times the final concentrations was applied in P_i/NaCl to the coated wells. Adhesion activities were measured with 2.5×10^4 cells.

The kinetics of fucosyltransferase-mediated cell adhesion seem to show a lag period in cell spreading of about 10–15 min when studied at 37°C with different concentrations of transferase used for the preparation of the surfaces. In this regard, adhesion on the transferase surfaces is different from that on plant lectin surfaces, which gives a more rapid reaction. Previously, fibronectin and glycosidases had been found to display a sigmoidal behavior when the numbers of attached cells were plotted as a function of time (6). Thus, the transferase-mediated adhesion might resemble physiological cell adhesion phenomena (18) more so than the adhesion on plant lectin.

Besides the human embryonic skin fibroblasts, 3T3 cells and undifferentiated teratocarcinoma cells were also able to spread on fucosyltransferase surfaces (unpublished data). This finding is to be expected because the acceptors containing Gal(β1-4)GlcNAc sequences are generally found in both glycoproteins and glycolipids from various sources (19). Interestingly, undifferentiated teratocarcinoma cells have been shown to express cell surface structures containing repeating Gal(β1-4)GlcNAc units that disappear during differentiation (20, 21). This change may explain the finding that the teratocarcinoma cells differentiated with retinoic acid attach and spread on fucosyltransferase surfaces to a lower extent than do undifferentiated cells (unpublished data). It was suggested recently that the carbohydrates of teratocarcinoma cells containing repeating Gal-GlcNAc units display glycosyltransferase-binding activities (21). That the adhesion-triggering activity may be a more general property of glycosyltransferases is also supported by the recent observation that a fucosyl(α1 → 2)transferase is able to mediate cell adhesion in a manner similar to the α1 → 3-α1 → 4 transferase (unpublished data).

Our preliminary data indicate that GDP-fucose acting as the fucose donor to the oligosaccharide may not be an efficient inhibitor of spreading. However, it is difficult to estimate the effect of GDP-fucose because cell-derived enzymes may destroy the sugar donor during the assay. However, it seems possible that the transferase could stimulate the cell transiently in

the presence of the nucleotide sugar, and this stimulation would give sufficient affinity for cell spreading. It should be emphasized that the cell adhesion mediated by many carbohydrate-reactive proteins becomes rapidly insensitive to the competing sugar after the initial stimulation by specific protein–sugar interactions (6).

Whether glycosyltransferases are expressed at the cell surface, as has been suggested (8, 21–24), in such amounts that they could trigger cell adhesion is of major importance at present. A high fucosyltransferase activity has been found in a cell line resistant to wheat germ lectin (25). Therefore, the possible cell surface exposure of this enzyme and its role in cell adhesion should be studied. Variations of hydrolase-transferase activities during differentiation, as suggested for the transferases by Shur and Roth (22), also are of interest.

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